Genetic Variation in the Endangered *Astragalus jaegerianus* (Fabaceae, Papilionoideae): A Geographically Restricted Species

George F. Walker[1] and Anthony E. Metcalf

Department of Biology, California State University San Bernardino, San Bernardino, California 92407, USA

Abstract.—Knowledge of genetic variation and population structure is critically important in the conservation of endangered species. The level and partitioning of genetic variation in the narrow endemic *Astragalus jaegerianus* was investigated using DNA sequence data and AFLP markers. The DNA sequence data for the cpDNA *trnL-F* and nrDNA ITS regions were monomorphic for *A. jaegerianus* but polymorphic for two congeners, which suggest *A. jaegerianus* is genetically depauperate. On the other hand, the genome-wide survey using AFLP markers revealed substantial gene diversity (0.2660) and significant population structure (global F_{ST} = 0.133, p < 0.01). This level of gene diversity and its partitioning among populations is comparable to patterns for geographically widespread species. These findings also challenge the hypothesis that levels of gene diversity are best predicted by population size. In this case, levels of gene diversity within populations are significantly correlated with population density, while geographic distances and gene flow explain patterns of population structure. These results suggest a breeding strategy for *A. jaegerianus* as a facultative outcrosser that relies more on outcrossing in areas of high plant density and less so in areas of low plant density. Conservation measures are recommended that include monitoring population genetics, numbers of individuals, and population densities.

Genetic variation is recognized as an important raw material of evolutionary and ecological processes in natural populations (Wright 1931; Karron et al. 1988; Clegg 1990; Epperson 1990; Frankham et al. 2002; Conner and Hartl 2004; but see Ackerly 2003). Effective conservation strategies for geographically restricted species require information about the level of genetic variation present and whether genetically distinct subpopulations exist. This information can aid management efforts by suggesting number of individuals and populations needed for the conservation of the species (Mace and Lande 1991; Lynch 1996; Nunney 2000; Frankham et al. 2002). The federally endangered *Astragalus jaegerianus* Munz (Fabaceae) is a narrow endemic of the Mojave Desert (southern California; USA) with small populations of relatively few individuals (U.S. Fish and Wildlife Service 2001). It is restricted within a range of 300 km^2 but only occupies approximately 85 km^2 of that habitat. In an intensive survey conducted in 2001, this species had an estimated population size of 5,700 plants (U.S. Fish and Wildlife Service 2001; Charis Professional Services Corporation [Charis] 2002; M. Hessing, Charis, unpublished data). To assist conservation efforts for *Astragalus jaegerianus*, we

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[1] Email: gwalker@sheridan.edu Present address: Sheridan College, P.O. Box 1500, 3059 Coffeen Ave., Sheridan, WY 82801 USA

investigated the level of genetic variation and its partitioning within the range of this species.

Population genetic theory predicts that geographically widespread plant species with large populations should typically possess higher levels of genetic variation than do geographically restricted species with small populations (reviewed in Hamrick et al. 1979; Hamrick and Godt 1989; Karron 1991; Soltis and Soltis 1991; Godt et al. 1996; Frankham et al. 2002; Avise 2004). This is predicated on the premise that species with small populations and few individuals are subject to genetic drift that reduces genetic variation (Barrett and Kohn 1991; Ellstrand and Elam 1993). Other factors affecting low genetic variation in restricted species may include strong directional selection and increased inbreeding (Karron 1991). While such reviews provide valuable comparisons of levels of genetic variation among widespread and restricted species, the effects of rarity on genetic variability may be confounded by other variables, such as historic demographic changes in population size, the lack of shared evolutionary histories, and sampling at dissimilar spatial scales (Karron 1987b; Gitzendanner and Soltis 2000; Cole 2003). For instance, in their review of the literature on levels of genetic variability between congeners, Gitzendanner and Soltis (2000) did find a pattern of higher levels of genetic variation for geographically widespread species than their geographically restricted congeners. But, they also note that there is a wide variance in the range of genetic variation among geographically restricted species, such that some restricted species exhibit as high or higher levels of genetic variation as their widespread congeners, while some widespread species exhibit low levels of genetic variation similar to that found in their restricted congeners (see also Karron 1987b; Karron et al. 1988; Soltis and Soltis 1991; Frankham 1995; Avise 2004).

Concerning historic demographic changes in populations, Karron (1987b; Karron et al. 1988) compared levels of genetic variation (allozyme variants) in geographically restricted and widespread vascular plant species across eleven genera that included *Astragalus* and found that while geographically restricted species tend to have lower levels of genetic variation than widespread species, there are exceptions. Additionally, Travis et al. (1996) examined AFLP variation in populations of the endangered sentry milk vetch (*Astragalus cremnophylax* Barneby var. *cremnophylax*) on the north and south rims of the Grand Canyon, and found that north rim populations possess a level of genetic variation consistent with historical levels, while the south rim populations possess almost no genetic variation. In both of these studies, the authors hypothesized that such heterogeneity in levels of genetic variation among geographically restricted species may be partially attributed to factors affecting reduced range size during the recent past. However, in the case of *A. jaegerianus,* there is no evidence of a historic range reduction (Munz 1941; Barneby 1964; U.S. Fish and Wildlife Service 2001; Charis 2002).

Population genetic theory also predicts that narrow endemic species with small populations and few individuals should exhibit strong population structure due to a higher proportion of genetic variation partitioned among populations (Hamrick and Godt 1989; Hamrick et al. 1991; Frankham et al. 2002; Avise 2004). However, Hamrick and Godt's (1989) review of the allozyme literature revealed that, while some geographically widespread species typically have a greater proportion of genetic variation partitioned within populations, there is no significant difference between geographically widespread and restricted species in how genetic variation is partitioned. In contrast, theory predicts that species with restricted ranges and small populations are expected to

have a greater proportion of genetic variation partitioned among populations (Karron 1991; Frankham et al. 2002; Avise 2004).

In this study, we tested the hypotheses that levels of genetic variation in species of restricted ranges and few individuals should be low, with a significant proportion of this variation partitioned among populations. We also examined whether the genetic structure of *Astragalus jaegerianus* correlates with its population geographic distribution. Such an estimate of genetic variation and population structure provides a basis for sustainable management of *Astragalus jaegerianus*.

Methods

The species.—Little is known of the biology of *A. jaegerianus* or its breeding system. It is a cryptic perennial that is generally found associated with a host or nurse shrub that it might use as a trellis (Gibson et al. 1998; Prigge et al. 2000; U.S. Fish and Wildlife Service 2001). It is a member of the Mojave Creosote Bush Scrub within its restricted range, but is only associated with shallow granitic or sandy soils (U.S. Fish and Wildlife Service 2001). Individual plants are often found in small, localized groups of a few individuals with intervening distances (often several hundred meters) of unoccupied, but suitable habitat (Charis 2002). *Astragalus jaegerianus* possesses the typical floral morphology characteristic of the subfamily Papilionoideae (Judd et al. 2002; Tucker 2003). Most species within the Papilionoideae are known to have showy, perfect flowers, which typically attract pollinators (Arroyo 1981). However, breeding systems vary within the Papilionoideae from obligate selfers to obligate outcrossers, including species capable of both (Arroyo 1981; Juan et al. 2004).

Population sampling.—The range of *Astragalus jaegerianus* can be subdivided geographically into five putative distinct populations (Figure 1; Coolgardie, Lane Mountain, Prospectors Wash, Brinkman Wash, and Goldstone). This follows population delineation in the Charis (2002) report and the U.S. Fish and Wildlife Service Recovery Plan (2001), with the exception of the Lane Mountain and Coolgardie populations. In the Charis' (2002) report, these two populations were considered as one (Coolgardie Mesa population), but for this study were split by the Copper City Road for the following reasons. The 2001 survey (Charis 2002) recorded an expanse of unoccupied habitat within the center of the Coolgardie Mesa population, which they attributed to deep alluvial soils but has also been heavily impacted by off-road vehicle users and campers, and residential and agriculture development. Plants recorded in the 2001 survey adjacent to the Copper City Road are approximately one kilometer apart from those on the other side of the road.

Our study began in 2001, one of the driest years on record for the Mojave Desert in California. Because there was essentially no growing season that year, we were only able to collect necrotic tissue. In subsequent years (2002 and 2003), our sampling of *Astragalus jaegerianus* tissue did include green leaflets. Twenty plants were sampled (sampling locations and UTM coordinates are available upon request) within each of the five putative populations. Sampling localities within each population varied from two in the Goldstone population to six in the Coolgardie population, which reflect the distribution of plants. Within each locality, two to 10 plants were collected from 10 to 30 meters apart. Because high molecular weight DNA is necessary for accurate and reproducible AFLP results (Ritland and Ritland 2000; Kingston and Rosel 2004), it was impossible to produce both sequence data and AFLP markers for the same subset of individuals. This was because from the necrotic stem tissue collected at the beginning of our study, we were
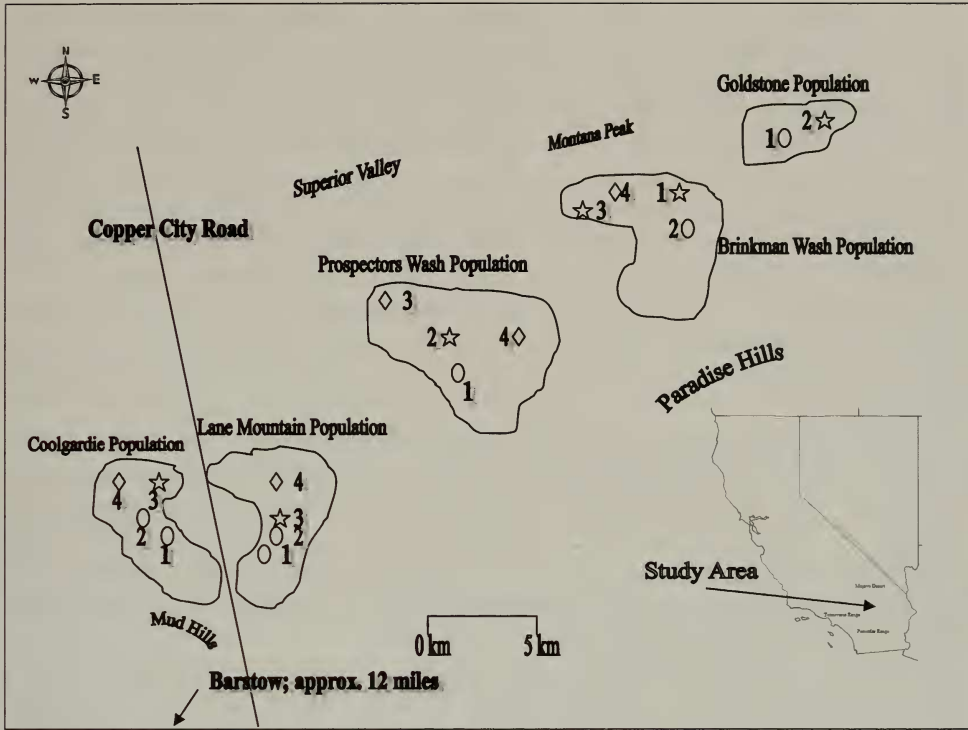
Fig. 1. Map of the known distribution of *Astragalus jaegerianus*. The study area is located approximately 12 miles NNE from Barstow, and is bounded on the north and east sides by Ft. Irwin Military Reservation and China Lakes Naval Weapons Center. Extent of the range of *Astragalus jaegerianus* is based on the Charis (2002) report. Plant collecting localities are delineated by: ○-DNA sequenced individuals only, ◇-AFLP Genotyped individuals only, ☆-both DNA sequenced and AFLP Genotyped individuals.

only able to isolate degraded DNA. Although we were able to amplify and sequence our loci of interest from this degraded DNA, the necrotic tissue did not provide sufficient high molecular weight DNA for generation of reliable AFLP markers. Thus, using the earlier collected tissue, nine individuals from each population were DNA sequenced, while later in the study ten individuals from each population were AFLP genotyped using green leaflet tissue collected in 2002–03 that yielded high molecular weight DNA; three individuals each that were collected within the Coolgardie, Brinkman Wash, and Goldstone populations were both DNA sequenced and AFLP genotyped. Two geographically widespread congeners (*Astragalus didymocarpus* H. & A. and *A. layneae* Greene) were also sampled within the range of *A. jaegerianus* (five of each species) for DNA sequencing to test the validity of the monomorphic sequence data for the 45 *A. jaegerianus* individuals.

Genomic DNA isolation.—Three different methods were used for genomic DNA isolation from plant tissue-Qiagen's DNeasy Mini Plant Kit (Chatsworth, California USA), Epicentre's MasterPure™ Plant Leaf DNA Purification Kit (Madison, Wisconsin USA), and a 2X CTAB (hexadecyltrimethylammonium bromide) extraction protocol (Doyle and Doyle 1987). Extracted genomic DNA was quantified using spectrophotometry and visualized for high molecular weight DNA by agarose gel (0.8%) electrophoresis.

PCR amplification and DNA sequencing.—To evaluate intraspecific sequence variation, loci of approximately 1300 total base pairs were selected from both the nuclear genome (nrDNA ITS region) and the chloroplast genome (cpDNA *trnL-F* region, which includes the *trnL* intron and 3′ exon, and the *trnL-F* spacer). To amplify the cpDNA *trnL-F* region, primers B-49317 (5′-CGAAATCGGTAGACGCTACG-3′) and A-50272 (5′-ATTTGAACTGGTGACACGAG-3′) developed by Taberlet et al. (1991) were used. To amplify the nrDNA region, primers ITS-4 (5′-TCCTCCGCTTATTGA-TATGC-3′) and ITS-5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) were used (White et al. 1989). In preparation for sequencing, a tailed primer method was utilized to generate amplicons by the Polymerase Chain Reaction (PCR; Mullis et al. 1986). This method employs a two-part primer system with the standard sequencing primers M-13-Forward or M-13-Reverse attached to the 5′ end of each PCR primer in a primer combination.

Amplification was performed using Eppendorf's (Hamburg, Germany) reagents provided with their TAQ polymerase, 1.0–2.0 µl of genomic DNA (a range of 5 to 100 ng/µl), 1.0 µl of each primer at 20 mMol, and 2.5 units of TAQ polymerase, assembled in 50 µl reactions. Cycle parameters were one to two minutes of initial denaturation at 94°C; 30 to 35 cycles of one minute at 94°C for denaturation, one minute at 48°C for annealing the primers, and one minute at 68°C for strand elongation; and five minutes at 68°C for final elongation.

Bi-directional cycle sequencing was accomplished on a Li-Cor, Inc. (Lincoln, Nebraska USA), 4300 DNA Analyzer using IRDyed labeled M-13 forward and reverse sequencing primers for nine individuals from each population with Epicentre's (Madison, Wisconsin USA) SequiTherm Excel™ II DNA Sequencing Kit-LC. Cycle sequencing parameters were an initial denaturation of 95°C for five minutes, and 20 cycles of 95°C for 60 seconds, 54°C for 30 seconds, and 70°C for 60 seconds. Sequences were deposited in the National Institute of Health's GenBank Database (Accession Numbers DQ403838–DQ403850).

Generation of AFLP markers.—AFLP analysis was executed for ten individuals from each population using Li-Cor's, Inc. (Lincoln, Nebraska USA) 4300 DNA Analyzer with their IRDye™ Fluorescent AFLP® Kit for Large Plant Genome Analysis, with several modifications to the kit's protocol. After several trials at various concentrations of genomic DNA, it was found that using approximately 500 ng of genomic DNA resulted in sharp and highly reproducible banding patterns in the gel images. This is similar to what Lanteri et al. (2004) found in their test of reproducibility of bands.

Other modifications included adding 2% deionized formamide to the PCR amplification reaction mixes to sharpen bands and reduce background noise, adjusting the pH of the adaptor ligation mix to pH 8.0 using 0.8 Mol Tris (pH 8.0), and ligating the adaptors at 16°C overnight (Berres 2002).

Sixty-four primer pairs were screened for band clarity and reproducibility, with four primer pairs (Mse1-CAC and EcoR1-ACT, Mse1-CAC and EcoR1-ACC, Mse1-CAC and EcoR1-ACA, Mse1-CAC and EcoR1-AGC) selected that produced clear interpretable and reproducible bands. Each primer pair for all 50 individuals was constructed in a single master mix, with tests for reproducibility of AFLP banding patterns conducted on a subset of the samples. Each individual of the subset was replicated for the four selected primer pairs twice, with a mean scoring error rate of less than one percent (Winfield et al. 1998).

Gels were scored using Li-Cor's, Inc., Saga™ automated AFLP Analysis Software (version 3.1), and verified by eye. Each band position was considered a locus with two alleles, and scored as either present or absent. Only bands occurring between 50 and 500 bases in length were scored, as size homoplasy resulting from co-migrating, non-homologous bands is more frequent among shorter and longer AFLP fragments (Vekemans et al. 2002).

Gene diversity.—Each population contained from two to six sampling localities, but plants from only three sampling localities within each population were used to generate AFLP markers except for one population. The Goldstone population contained two sampling localities, with plants collected from one locality used to generate AFLP markers. Population parameters used to characterize gene diversity included the percentage of polymorphic loci, the number of AFLP phenotypes, and Nei's (1978; 1987) gene diversity. ARLEQUIN 2.0 (Schneider et al. 2000) was used to derive the percentage of polymorphic loci and the number of AFLP genotypes within each population, while POPGENE 1.31 (Yeh et al. 1999), was used to estimate Nei's (1978; 1987) gene diversity across the range of the species and within each population (Gaudeul et al. 2000).

Population genetic structure and gene flow.—The distribution of genetic variation was assessed by a hierarchical analysis of molecular variance (AMOVA, Excoffier et al. 1992) at two levels-within populations and among populations. Wright's (1951) global F_{ST} and population pairwise F_{ST} coefficients were estimated using AMOVA, with significance evaluated by use of a randomization procedure using 1034 randomization replicates and assuming Hardy-Weinberg equilibrium (Gaudeul et al. 2000). The software program Tools for Population Genetics Analysis (TFPGA; Miller 2000) was used to estimate θ_{ST} (Weir and Cockerham 1984; Weir 1996), while POPGENE 1.31 was used to estimate Neiv's G_{ST} (Nei 1987); both are analogs to Wright's (1951) F_{ST}. TFPGA was also used to calculate Nei's unbiased (1978) genetic distances among the five populations, and to construct a population UPGMA cluster phenogram based on the population pairwise comparisons of the genetic distances. The constructed tree was bootstrapped (1000 permutations) to determine support for each node.

Population genetic structure was tested by constructing an UPGMA phenogram to portray genetic relationships among individuals using the software package PAUP 4.0d64 (Swofford 1998). Principle Coordinates Analysis (PCoA) using AFLP phenotypes was performed using the R Package, version 4.0d6 (Casgrain and Legendre 2001) that allows the positioning of objects in a two-dimensional reduced space that preserves their relationships.

To test whether population structure follows an isolation by distance model, two distance matrices were compared (Mantel test; Mantel 1967) using the R Package, version 4.0d6 (Casgrain and Legendre 2001), with significance (r_M) tested using 9999 permutations. In this case, we tested for correlation between Nei's genetic distances and geographic distances among all five populations. Geographic distances between populations were determined by measuring a straight line between approximate central points within each population (Legendre and Legendre 1998).

Tests for association between population density and gene diversity, population area and gene diversity, and population size and gene diversity were performed in SPSS (Windows version 12.0, SPSS Inc., Chicago, Illinois USA). Population demographic values are from Charis (2002) (see Table 1). For all tests of association performed in

Table 1. Population demographics and gene diversity statistics. Descriptive statistics for genetic variability include the proportion of polymorphic loci (P), the number of AFLP phenotypes for each population with the number of individual plants sampled in parentheses, mean distance between sampling localities within each population, and Nei's (1978) index of gene diversity.

Population	Sample Size	Pop.[a] Size	Pop.[b] Range	Pop.[c] Density	P(%)	No. of AFLP phenotypes	MSD[d] ± SE	Gene Diversity Mean ± std. dev.
Lane Mountain	10	1027[e]	4911	0.21	55.8	10 (10)	0.973 ± 0.133	0.1905 ± 0.2008
Coolgardie	10	987[e]	4867	0.20	55.5	10 (10)	1.041 ± 0.087	0.1910 ± 0.2012
Prospectors Wash	10	1667	4794	0.38	68.3	10 (10)	3.438 ± 0.453	0.2316 ± 0.2014
Brinkman Wash	9	1487	5497	0.27	65.5	9 (9)	2.815 ± 0.105	0.2283 ± 0.2036
Goldstone	10	555	1283	0.43	70.0	10 (10)	0.820 ± 0.000	0.2505 ± 0.2004

[a] Population sizes are taken from the Charis (2002) report.

[b] Total acreage in each population polygon (Charis 2002).

[c] Plants per acre.

[d] Mean Sampling Distance (km) was derived by computing the mean distance between each population's sampling points, and then computing the mean of means with standard error for each population.

[e] The Charis (2002) report considered the Lane Mountain and Coolgardie populations as one population (Coolgardie Mesa). Population size estimates were derived by proportioning the Coolgardie Mesa population size estimate between the proportions of acreage determined by Charis (2002) on either side of the Copper City Road.

SPSS, Pearson's product moment correlation coefficient (r) was derived at a significance level of $\alpha = 0.05$.

ARLEQUIN 2.0 (Schneider et al. 2000) was used to estimate gene flow among the five populations. Gene flow (Nm; $F_{ST} = 1/(4\ Nm + 1)$) was derived indirectly using the estimates of population pairwise F_{ST} values (Neigel 1997; Wright 1951), also derived in ARLEQUIN 2.0. A Mantel test for correlation (Mantel 1967) between Nm and geographic distances was performed using the R Package, version 4.0d6 (Casgrain and Legendre 2001).

Results

DNA sequencing.—The nrDNA ITS region and the cpDNA *trnL-F* region were sequenced for nine individual plants from each population (n = 45). The cpDNA *trnL-F* sequence produced an amplicon of 699 nucleotide bases, while the nrDNA ITS sequence produced an amplicon of 595 nucleotide bases. Sequences from each region were verified, with boundary determination, by comparison with retrieved sequence data for *Astragalus* on the National Institute of Health's GenBank Database (<http://www.ncbi.nlm.nih. gov/Genbank/>). Alignments of the cpDNA and nrDNA sequences derived from the 45 *A. jaegerianus* individuals failed to reveal any sequence variation within both regions.

On the other hand, the nrDNA ITS sequences for five *Astragalus didymocarpus* individuals revealed nine nucleotide polymorphisms out of 595 bases (1.5%, three genotypes), while DNA sequences for three *A. layneae* individuals revealed two nucleotide polymorphisms out of 595 bases (0.34%, three genotypes). For sequences of the cpDNA *trnL-F* region, three of five *A. didymocarpus* individuals possessed two indels (one 7 bases in length and the other one base only) along with three single nucleotide polymorphisms (substitutions) out of 699 bases (0.43%, three haplotypes). Three

Table 2. Analysis of Molecular Variance (AMOVA) for two hierarchical levels (among populations and within populations).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	4	343.516	5.26513	13.31
Within populations	44	1509.300	34.30227	86.69
Total	48	1852.816	39.56741	

$F_{ST} = 0.133$, p < 0.00001

individuals of *A. layneae* were sequenced for the same region with two nucleotide polymorphisms out of 699 bases (0.29%, two haplotypes) and no indels. While the sequence data characterized the 45 individuals of *Astragalus jaegerianus* as monomorphic, the variable sequence data for individuals of *Astragalus didymocarpus* and *A. layneae*, combined with the demonstrated utility of the examined loci, suggests that this narrow endemic may be relatively depaurate of genetic variation compared to its congeners.

AFLP markers.—AFLP generated a total of 360 markers from the four primer pairs for 49 individuals (10 from each population, with the exception of the Brinkman Wash population, wherein one individual was omitted from the study due to lack of amplification in the selective amplification step). Of the 360 AFLP bands scored, 290 (80.5%) were polymorphic. The four AFLP binary data sets were combined after removal of the monomorphic bands to make a single data set for further analyses (Despres et al. 2003; Coart et al. 2005; Muellner et al. 2005).

Gene diversity.—The AFLP data reveals each *A. jaegerianus* individual sampled as a unique multi-locus genotype, which is contrary to the monomorphism found in the chloroplast and nuclear DNA sequences. The proportion of polymorphic loci found in each population ranged from a low of 55.5% in the Coolgardie population to a high of 70.0% in the Goldstone population (Table 1). Mean gene diversity (Nei 1987) within the range of the species was estimated at 0.2660 (SD = 0.1603). When estimated separately for the five populations, mean gene diversity ranged from a low of 0.1905 (SD = 0.2008) for the Lane Mountain population in the southwest to a high of 0.2505 (SD = 0.2004) for the Goldstone population in the northeast (Table 1).

Population genetic structure and gene flow.—Analysis of molecular variance (AMOVA) revealed that 13% of the observed genetic variation was partitioned among the five populations and 87% partitioned within populations (Table 2). Global F_{ST} (Wright, 1951) obtained by AMOVA revealed significant differentiation among the five populations ($F_{ST} = 0.133$, p < 0.01). Analogs of F_{ST} were similar but slightly higher (estimated $\theta_{ST} = 0.174$ and $G_{ST} = 0.180$). F_{ST} indices obtained for pairwise comparisons among the five populations ranged from a low of 0.051 (for Prospectors Wash/Brinkman Wash populations) to a high of 0.232 (for Lane Mountain/Goldstone populations), with significant differentiation observed for all population pairwise comparisons (Table 3).

Estimated unbiased genetic distances (Nei 1978; 1987) of pairwise comparisons among the five populations and geographic distances between populations are displayed in Table 4. In a Mantel test for isolation by distance, a significant positive correlation ($r_M = 0.823$, p < 0.05) was found between the population pairwise estimates of unbiased genetic distances and geographic distances (5.00 km to 27.50 km) separating each population. The population UPGMA dendrogram (Figure 2) based on Nei's unbiased genetic distances also supports this geographic structure. Both the unrooted UPGMA

Table 3. Population pairwise F_{ST} indices[a] derived in AMOVA below the diagonal and estimated numbers of migrants (m) exchanged among populations per generation above the diagonal [$F_{ST} = 1/(4\ Nm + 1)$].

Population	Ln. Mtn.	Coolg.	Prosp. Wash	Brinkm. Wash	Goldstone
Lane Mtn.	****	3.352	2.135	1.314	0.826
Coolgardie	0.06941	****	3.233	1.678	0.918
Prospectors W.	0.10482	0.07178	****	4.650	1.635
Brinkman W.	0.15990	0.12965	0.05102	****	1.977
Goldstone	0.23233	0.21408	0.13266	0.11228	****

[a] p<0.01 for all population pairwise F_{ST} indices (1024 permutations)

dendrogram of individuals (Figure 3) and the first and second principal coordinates of the PCoA cluster plot (accounting for 10.0 and 5.3% of the variance, respectively; Figure 4) illustrate this significant population structure in different ways. Within both figures, individuals tend to cluster with other individuals collected within the same population, with a few exceptions. Specifically, individuals collected within the Lane Mountain and Coolgardie populations cluster together in both the PCoA cluster plot and UPGMA dendrogram. This suggests a close relatedness between individuals collected from both populations, which is consistent with both populations having the lowest pairwise genetic distance (0.0381; Table 4). Even so, the populations show significant genetic differentiation (population pairwise F_{ST} coefficient 0.069, p < 0.001; Table 3), which supports delineating two populations in the southern end of the species range, rather than one population as was done in the Charis (2002) report.

There is a significant positive correlation between population density and gene diversity (r = 0.931, p < 0.05), but not between gene diversity and population size (r = −0.022, p > 0.05) or population area (r = −0.616, p > 0.05). This is contrary to predictions, as population area or population size is typically positively linked to the level of gene diversity in a population (Despres et al. 2002; Godt et al. 1996; Godt et al. 2005). For instance, individuals within the Goldstone population were spread over a relatively large proportion of the PCoA two-dimensional coordinate space (Figure 4) and had the highest gene diversity (0.2505; Table 1); yet, the Goldstone population is the smallest of the five populations-both in census numbers (555 individuals) and in area (1283 acres; Charis 2002). This suggests that in this case the level of gene diversity a population may possess is best predicted by plant density.

Estimated migration rates per generation (Nm; Table 3) ranged from a high of 4.65 between Prospectors Wash/Brinkman Wash populations to a low of 0.83 between Lane Mountain/Goldstone populations. The estimated migration rates were higher between

Table 4. Estimates of Nei's (1978) unbiased genetic distances between populations are displayed on the lower diagonal, and geographical distances[a] between populations are displayed on the upper diagonal.

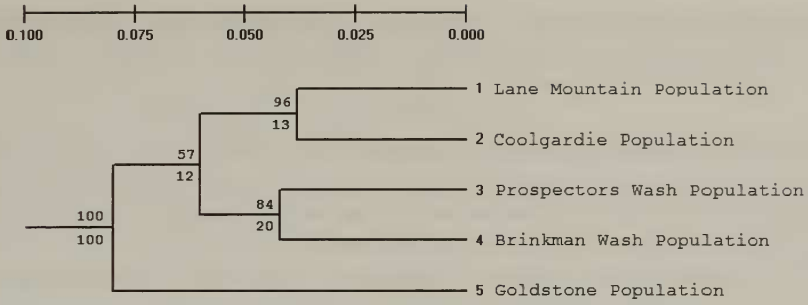| Population	Lane Mtn.	Coolgardie	Prospectors W.	Brinkman W.	Goldstone
Lane Mtn.	*****	5.00	10.50	18.00	23.00
Coolgardie	0.0381	*****	14.50	23.00	27.50
Prospectors W.	0.0537	0.0501	*****	9.50	14.50
Brinkman W.	0.0687	0.0680	0.0420	*****	5.50
Goldstone	0.1004	0.0926	0.0639	0.0619	*****

[a] Kilometers

Fig. 2. UPGMA cluster diagram derived from estimates of Nei's (1978) unbiased genetic distances among populations of *Astragalus jaegerianus*. Values above the nodes are bootstrap values supporting the node (1000 permutations), and values below the node are the proportion of loci supporting the node.
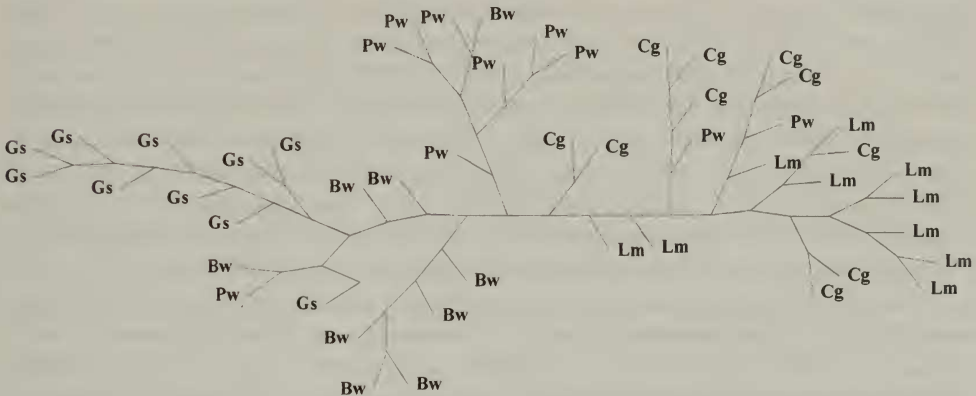

Fig. 3. Unrooted UPGMA dendrogram displaying genetic distances among the 49 individual *Astragalus jaegerianus* AFLP phenotypes. Population designations are Lm for Lane Mountain, Cg for Coolgardie, Ps for Prospectors Wash, Bw for Brinkman Wash, and Gs for Goldstone.
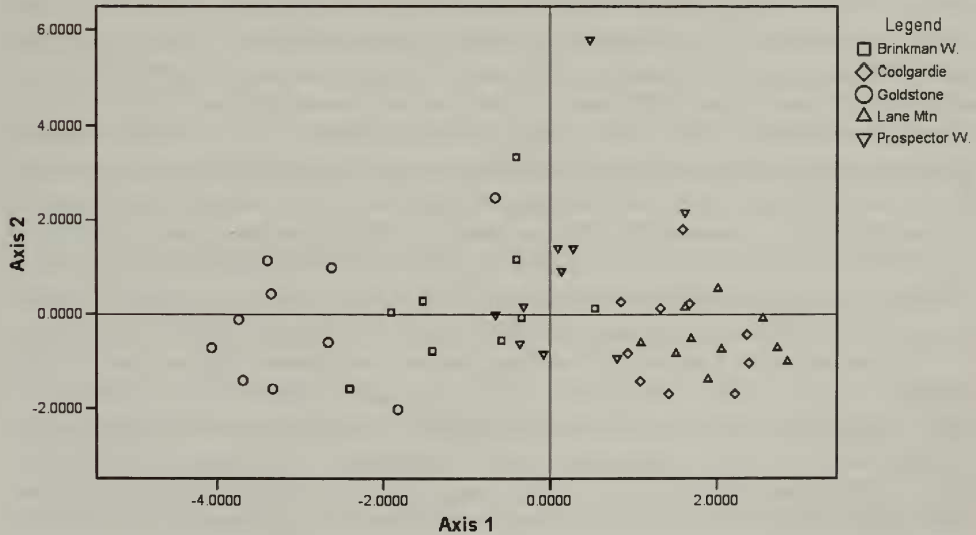
Fig. 4. PCoA cluster plot of the first and second principal coordinates based on the 49 individual *Astragalus jaegerianus* AFLP phenotypes.

adjacent populations than between populations separated by one or more populations and are negatively correlated with geographic distance ($r_M = -0.671$, $p < 0.05$).

Discussion

Gene diversity.—The objective of this study was to investigate the level of genetic variation and it's partitioning for *Astragalus jaegerianus*. We hypothesized that this narrow endemic, with small populations and few individuals, would be characterized with low genetic variation partitioned at the population level. Both data sets (DNA sequences and AFLP markers) gave seemingly incongruent results. No genetic variation was observed in the examined sequences of the chloroplast and nuclear genomes, while AFLP analysis of the entire genome (see Vos et al. 1995; Mueller and Wolfenbarger 1999) revealed substantial genetic variation and population structure. This lack of DNA sequence variation may be due to *A. jaegerianus'* small population sizes and restricted geographical range, or because of unknown historical factors (Karron et al. 1988; Schmidt and Jensen 2000). In comparison, sequence data from *Astragalus didymocarpus* and *A. laynae* did reveal substantial DNA sequence variation for both the same chloroplast and nuclear loci. These same loci have also been utilized to characterize intraspecific genetic variation within other plant taxa (Terry et al. 2000; Chiang et al. 2004; Dobes et al. 2004; Franzke et al. 2004; Lihova et al. 2004; Ruggiero and Procaccini 2004; but see Shaw et al. 2005). Therefore, when only DNA sequence variation at these loci is considered, this narrow endemic appears genetically depauperate, and suggests that *A. jaegerianus'* populations have recently descended from a few individuals.

In contrast, the genome-wide survey using AFLP markers revealed a level of gene diversity for *A. jaegerianus* that is similar to levels of gene diversity observed in other studies that used AFLP markers to characterize gene diversity in geographically widespread species. Despres et al. (2002) characterized the levels and distribution of genetic variation for the widespread European globeflower (*Trollius europaeus* L.) and found that levels of genetic variation among the three regions ranged from 0.158 to 0.229, which is also similar to the level of gene diversity (0.243) that Tang et al. (2003) reported in their study of the geographically widespread *Hibiscus tiliaceus* L. (Malvaceae). The level of gene diversity for geographically restricted species is different. Juan et al. (2004), when characterizing the levels of AFLP gene diversity for the endangered *Medicago citrine* (Font Quer) Greuter (Fabaceae, Papilionoideae), found that gene diversity ranged from 0.035 to 0.143, a ~13 to 55% reduction from those gene diversity indices reported here for *A. jaegerianus*. Travis et al. (1996) report an extremely low level of gene diversity (0.0177 and 0.0373) for the south rim populations of the critically endangered *Astragalus cremnophylax* var. *cremnophylax*. The patterns of AFLP gene diversities reported for geographically restricted species (Travis et al. 1996; Juan et al. 2004) and geographically widespread species (Despres et al. 2002; Tang et al. 2003) are similar to patterns of gene diversity for restricted and widespread species reported in Hamrick's et al. (1991) review of the allozyme literature. In their review, they note a pattern of gene diversity that averages around 0.202 for widespread species and around 0.09 for narrow endemics. The level of gene diversity for *A. jaegerianus* is higher than expected when compared to these patterns (Karron 1991), as population genetic theory predicts that species with restricted ranges and few individuals would have low levels of genetic variation (Hamrick & Godt 1989; Hamrick et al. 1991; Soltis and Soltis 1991; Ellstrand and Elam 1993; Godt et al. 1996; Gitzendanner and Soltis 2000; Frankham et al. 2002; Avise 2004). Although comparing patterns of gene diversity generated by either allozyme data (co-dominant

markers) or AFLP data (dominant markers) may not be equitable, it does provide a comparison for putting the level of gene diversity found in *A. jaegerianus* in context with gene diversity patterns reported for other plant taxa using different molecular markers (Alexander et al. 2004; Nybom 2004).

This high level of genetic diversity for this narrow endemic, with small populations and few individuals, suggests that *Astragalus jaegerianus* has undergone a recent population contraction or is undergoing population contraction, such that the populations may have had a recent common history (Schmidt and Jensen 2000; Ellis et al. 2006; Vilatersana et al. 2007). Similar high levels of gene diversity for narrow endemic plants have been observed previously (Karron 1987b; Alexander et al. 2004; Vilatersana et al. 2007). Karron (1987b; Karron et al. 1988) compared levels of allozyme genetic variation in both restricted and geographic widespread plant congeners including four species of *Astragalus*, and observed that there is considerable variance in levels of gene diversity reported for rare species. In a subsequent review, Karron (1991) observed that in some cases the extent of geographic range could be a poor predictor of the level of genetic variation a species may possess (see also Soltis and Soltis 1991). Alexander et al. (2004) reported a level of population gene diversity (ranging from 0.14 to 0.18) for the narrow endemic *Astragalus oniciformis* Barneby that is a little lower but similar to what was found for *A. jaegerianus* (Table 1). Vilatersana et al. (2007) also reports high gene diversity (0.17) for the endangered *Femeniasia balearica* (J. J. Rodr.) Susanna-a narrow endemic confined to the Balearic Islands of Spain. Both Alexander et al. (2004) and Vilatersana et al. (2007) attributed their high gene diversity to either recent change in population demographics, reduced effects of genetic drift on populations of long-lived woody species with overlapping generations, or groups of putative identified populations acting as a population. Other factors influencing the observed patterns of genetic variation in these geographically restricted species may include differences in the present and historic range of a species, recent bottlenecks and founder events, and repeated cycles of bottlenecks and range expansions (Karron 1991; Schmidt and Jensen 2000; Frankham et al. 2002; Avise 2004). However in our case, we have no information regarding these factors for *A. jaegerianus*, except that it has been restricted in range and population size since its discovery in 1938 (Munz 1941; Barneby 1964).

Typically within plant taxa there is a positive correlation between geographic range and gene diversity or population size and gene diversity (Hamrick & Godt 1989; Ellstrand & Elam 1993; Godt et al. 1996; Fischer & Matthies 1998; Gram & Sork 1999; Gaudeul et al. 2000; Lu et al. 2005), both of which are commensurate with population genetic theory that small populations cannot maintain high levels of gene diversity (reviewed in Soltis & Soltis 1991). Our findings demonstrate that population density ($r = 0.931$, $p < 0.05$), rather than population size ($r = -0.022$, $p > 0.05$) or population area ($r = -0.616$, $p > 0.05$), is a better predictor for the levels of genetic variation possessed by any of the five populations of *Astragalus jaegerianus*. This association between population density and gene diversity suggests a reproductive strategy for *A. jaegerianus* (discussed below).

Population genetic structure and gene flow.—The partitioning of genetic variation among populations by *Astragalus jaegerianus* is noteworthy, as all five populations are small, both in geographic area and census size; a pattern that led us to predict that this narrowly endemic species would possess low genetic variation partitioned primarily at the population level (Hamrick and Godt 1989; Hamrick et al. 1991; Soltis and Soltis 1991; Ellstrand and Elam 1993). The proportion (13%) of genetic variation partitioned among populations of *A. jaegerianus* is less than the amount of genetic variation partitioned

among populations of geographically restricted species, and more similar to patterns reported for geographically widespread species (Hamrick et al. 1991; Soltis and Soltis 1991). In AFLP studies in particular, Travis et al. (1996) reported that 73% of the observed genetic variation for the endangered *Astragalus cremnophylax* var. *cremnophylax* was partitioned among populations, while Juan et al. (2004) found 44% of the AFLP variation reported for the endangered *Medicago citrine* partitioned among populations. A specific example of genetic variation (AFLP) partitioning by a geographically widespread species is the European globeflower, wherein Despres et al. (2002) found this species partitions its genetic variation at 16% among populations across the Alps, 31% among populations throughout the Pyrenees, and 26% in populations across the Fennoscandia region; values, although higher, are more within the range of that observed for *A. jaegerianus* (13%). This suggests, along with gene diversity indices, that *A. jaegerianus* and its populations may be experiencing range and/or population contractions (Schmidt and Jensen 2000; Ellis et al. 2006).

The genetic structure of *Astragalus jaegerianus* corresponds with the species' spatial arrangement of populations in the field, which follows a near linear pattern from southwest to northeast (Figure 1). There is a linear trend from the southwest to the northeast for increasing genetic distances, and increasing population differentiation; a geographical pattern that is unlikely a result of chance or random processes. While the Mantel test of correlation between genetic distance and geographic distance matrices supports an isolation by distance model for the species, the linear patterns of population pairwise comparisons of F_{ST} indices (Table 3) and genetic distances (Table 4) also suggest that the geographic arrangement of the populations follows the one-dimensional stepping stone model, rather than the island model for gene flow (see the review in Halliburton 2004). This spatial arrangement of the populations on the landscape combined with the patterns of genetic variation within and among populations and estimates of gene flow among populations (below) all provide insight into the reproductive biology of *Astragalus jaegerianus*.

The indirect estimates of gene flow partially explain population genetic structure and population levels of genetic variation observed for *Astragalus jaegerianus* (Wright, 1951; reviewed by Slatkin 1985; but see Bossart and Powell 1998; Whitlock & McCauley 1999). Typically, a higher proportion of genetic variation is partitioned within populations of geographically widespread and long-lived species-species often with the potential for long-range gene flow; whereas in species with restricted ranges, small populations, and limited gene flow, a higher proportion of the genetic variation is partitioned among populations (Hamrick and Godt 1989; Karron 1991; Richards et al. 1999). It has already been shown that *A. jaegerianus* possesses a level of gene diversity and population genetic structure comparable to patterns observed in geographically widespread species, thus the estimated high gene flow (*Nm*; Table 3) for *A. jaegerianus* is not unexpected and is comparable to other studies with similar population genetics (Despres et al. 2002; Tang et al. 2003; Alexander et al. 2004).

The analyses support a mixed breeding strategy of outcrossing and selfing that suggests pollinator-mediated gene flow. We hypothesize that within areas of high plant density *A. jaegerianus* may rely on outcrossing facilitated by one or more pollinators (Levin & Kerster 1969a; Levin & Kerster 1969b; Ellstrand et al. 1978; Van Treuren et al. 1993; Franceschinelli and Bawa 2000). This is based on floral morphology and the association of population gene diversity indices scaling with population density, as well as the global and population pairwise F_{ST} coefficients (Table 2 and 3). As such, we predict greater gene

diversity within higher density populations, with less genetic differentiation among adjacent populations than more distant populations due to gene flow. Regarding the distribution of genetic variation among plants with mixed mating systems, Loveless and Hamrick (1984) and Hamrick and Godt (1989) suggest that an allozyme-generated F_{ST} coefficient of ~0.2 indicates outcrossing is predominantly occurring, while a F_{ST} coefficient of ~0.5 indicates selfing is predominantly occurring. Additionally, gene flow mediated by pollinators among most members of the Papilionoideae is supported by showy, perfect flowers, which typically attract pollinators (Arroyo 1981). However, breeding systems vary within the Papilionoideae from obligate selfers (geitonogamy and autogamy) to obligate outcrossers (cross pollination between flowers from different plants), with some Papilionoideae taxa, including some *Astragalus* species, capable of both (Karron 1987a; Arroyo 1981; Juan et al. 2004). Moreover, geographic distance and population density have been shown to interact to affect the rate of gene flow between populations (Bateman 1947; Karron et al. 1995; Roll et al. 1997; Richards et al. 1999). Roll et al. (1997) experimentally demonstrated that pollinator visitation at flowers increases as floral density increases within a population. For populations with high floral densities, pollinator visits are high but mean flight distances are shorter, while in low-density populations pollinator visits are low with longer mean flights distances (Roll et al. 1997; Richards et al. 1999). Thus, in high-density populations, plants receive more pollinator visits than plants in lower density populations, which effectively increase the effective local pollen pool among plants, and maintains higher levels of within-population gene diversity. On the other hand, in low-density populations plants receive fewer pollinator visits, which results in a reduced effective pollen pool and a greater reliance on self-fertilization for seed production, both of which can decrease within-population gene diversity (Proctor et al. 1996). Of interest is that gene flow is likely to be higher among low-density populations due to fewer within-population flower visits and increased pollinator flight distances (Handel 1983). However, if selfing increases in low-density populations, we predict lower within-population gene diversity. These are the patterns we observed.

Summary.—*Astragalus jaegerianus* possesses a high level of gene diversity as estimated by AFLP that is predominantly partitioned within populations; a scenario comparable to patterns of gene diversity observed in geographically widespread species. The data indicate that there is low but significant genetic structure, with each population genetically divergent from the others. While population density partially predicts the level of genetic variation within populations, genetic structure among populations is explained by geographic distances, suggesting a stepping-stone model for gene flow. These results support *A. jaegerianus* as a facultative outcrosser with pollinator-mediated gene flow. The association of gene diversity and population density is also congruent with both outcrossing and selfing, with less dense populations having a higher proportion of selfers. In the case of *Astragalus jaegerianus*, this also explains why gene diversity is not correlated with population size. This is noteworthy, as normally population size and gene diversity are linked (Fischer & Matthies 1998; Gaudeul et al. 2000; Despres et al. 2002).

Conservation implications and recommendations.—The ultimate goal of conservation biology is to conserve species and their evolutionary potentials. The monomorphic DNA sequence data, considered alone, suggest that an estimated effective population size for *Astragalus jaegerianus* may be far below the 2001 census population size of approximately 5,700 individuals (Frankham et al. 2002; Halliburton 2004). This suggests management efforts should focus on conserving as many individuals as possible across the range of the species to capture any variation present in other gene loci.

On the other hand, the AFLP analyses of the levels and distribution of genome-wide variation indicates that *A. jaegerianus* possesses a level of gene diversity and population structure comparable with geographically widespread species; a scenario that suggests this species has undergone, or is undergoing, population contraction. Each population may contain some unique genetic contribution that is locally adapted to environmental conditions, and, since gene flow appears high among adjacent populations, augmentation for any of the five populations appears unwarranted at this time (Ellstrand and Elam 1993). Goldstone, the smallest of the five populations, contains the highest gene diversity. However, because of its small size, both in acreage and census numbers, it is the most at risk of the five populations from habitat loss and degradation.

The hypothesis of *A. jaegerianus* being a facultative outcrosser should be experimentally tested in the field. In addition, pollinators of *A. jaegerianus* must be identified, and their reproductive biology considered during conservation planning. One or more factors of the reproductive biology of pollinators may be a limiting factor for the conservation of *A. jaegerianus*, and maintenance of the present levels and partitioning of genetic variation (Karron 1989).

Lastly, this study provides important baseline data for monitoring trends in the population genetics of *Astragalus jaegerianus*. Since *A. jaegerianus*' level of genetic variation and its partitioning among populations is dependent on population size and density, fluctuations in either of these parameters may put this species at risk. Monitoring efforts, therefore, should include regular plant surveys to estimate population sizes and plant densities, as well as periodic surveys using molecular assays to assess changes in levels of genetic variation and population genetic structure. Future population genetic work on *A. jaegerianus* should include a comparison of the population genetics between the three species of *Astragalus* (*A. jaegerianus*, *A. didymocarpus*, and *A. Laynae*) and testing the validity of the five putative populations, with a fine-scale study utilizing a larger sample size.

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